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Award Number: W81XWH-06-1-0181

TITLE: Analysis of Ethnic Admixture in Prostate Cancer

PRINCIPAL INVESTIGATOR: Cathryn Bock, Ph.D.

CONTRACTING ORGANIZATION: Wayne State University
Detroit, MI 48202

REPORT DATE: December 2007

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 01-12-2007		2. REPORT TYPE Final		3. DATES COVERED 30 NOV 2005 - 29 NOV 2007	
4. TITLE AND SUBTITLE Analysis of Ethnic Admixture in Prostate Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-06-1-0181	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Cathryn Bock, Ph.D. Email: bockc@med.wayne.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Wayne State University Detroit, MI 48202				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Evidence for a genetic component to prostate cancer is strong, however few genes have been identified, and most of the genetic risk remains undefined. To date, multiple traditional genome scans have been performed, and several susceptibility loci have been identified. Traditional genome scans using familial data have generally not included enough African Americans to provide adequate statistical power to detect linkage. Our project uses a novel approach to gene discovery with greater power to detect genetic effects, admixture mapping, to identify prostate cancer susceptibility loci in a sample of African American men. Freedman et al. reported a susceptibility region on chromosome 8q24, detected by admixture mapping in 1,597 African American men. In the current study, approximately 800 samples from 2 case-control study of prostate cancer were genotyped for ancestry informative markers across the genome. Admixture mapping analyses were performed using ADMIXMAP and ANCESTRYMAP statistical programs. The prostate cancer susceptibility locus on 8q24 identified by Freedman et al. was confirmed, with a peak lod score estimated using ANCESTRYMAP of 1.54 between markers rs12547950 and rs4367565. A new prostate cancer susceptibility locus on chromosome 5q35 was identified, with a maximum lod score of 3.16 detected at marker rs7729084 using ADMIXMAP. This new region is actively being explored via fine mapping and candidate gene approaches.					
15. SUBJECT TERMS Prostate cancer, admixture mapping					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	10	19b. TELEPHONE NUMBER (include area code)

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INTRODUCTION:

Evidence for a genetic component to prostate cancer is strong, however few genes have been identified, and most of the genetic risk remains undefined. To date, multiple traditional genome scans and linkage analyses have been performed, and several susceptibility loci and candidate genes have been identified, including *HPC1*, *HPCX*, *HPC20*, *CAPB*, *PCAP*, *RNASEL*, *HPC2/ELAC2*, and *MSR1*. Traditional genome scans using information from prostate cancer families, however, have generally not included enough African American families to provide adequate statistical power to detect linkage. The goal of this research proposal is to use a novel approach to gene discovery, admixture mapping, to identify potential prostate cancer susceptibility genes in a group of African American men. Admixture mapping has greater power to detect genetic effects than traditional genome linkage scans. Recently, Freedman et al. published results from an admixture mapping study of prostate cancer in 1,597 African American men which detected a susceptibility region on chromosome 8q24 (1). In the current study, approximately 800 samples from 2 case-control study of prostate cancer were genotyped for ancestry informative markers across the genome, using a similar marker panel to that used by Freedman et al. The admixture mapping analyses were performed using ADMIXMAP (2, 3) and ANCESTRYMAP (4) statistical programs. Regions showing strong linkage using the admixture mapping approach will be followed by future studies using fine mapping with a denser set of informative markers in the regions of interest and candidate gene studies.

BODY:

The details of progress within each Task are as follows.

Task 1. To obtain genotype information for all study subjects (Months 1-18):

- a. Prepare batches of DNA and ship to ParAllele, starting with samples from controls (Months 1-17).*
- b. ParAllele to perform Genotyping and transmit results to Dr. Bock (Months 2-18).*

DNA samples for 520 cases and 287 controls were prepared for genotyping, and shipped to the laboratory. In addition to the 351 cases and 95 controls from HFHS, we also included 456 cases and 192 controls from Dr. Rick Kittles' prostate cancer case control study (described in Bonilla et al., (5)), with IRB approval, thereby more than doubling our sample size. For quality control, DNA samples from 30 CEPH individuals were included so that their genotype results could be compared with those publicly available through HapMap.

Because ParAllele was out of business when we were ready to genotype, we used a panel of 1536 ancestry informative SNPs developed by David Reich at the Broad Institute for use on the Illumina BeadStation platform. Earlier versions of this panel were used in the Freedman et al. prostate cancer admixture mapping study (1). This panel has very high reliability and success rate in Dr. Reich's lab.

Genotyping was completed by the Wayne State University Genomics core using primers provided by David Reich and the Illumina BeadStation platform on all samples and the raw results were provided to the PI in February, 2007.

Task 2. To identify candidate prostate cancer susceptibility loci using mapping by admixture linkage (MALD) (Months 1-22).

- a. Set up database and preliminary ADMIXMAP program (Months 1-6).*
- b. Perform preliminary analyses and refine ADMIXMAP program (Months 6-18).*
- c. Calculate final LOD scores and 95% confidence intervals for regions that show possible linkage (Months 18-22).*
- d. Where necessary, extend the score test and likelihood ratio tests in ADMIXMAP to test for gene-environment interactions. (Months 20-22).*

The ADMIXMAP software was successfully set up on a local computer, and the PI successfully ran a test data set through the program. After some preliminary data cleaning, the data was sent to Dr. David Reich at the Broad Institute and he performed further data quality checks and performed admixture analysis using his ANCESTRYMAP program. After receiving the cleaned data back from Dr. Reich, we used the ADMIXMAP program to repeat the admixture analyses. ADMIXMAP is less conservative than ANCESTRYMAP, and therefore more likely to detect linked regions. The more conservative ANCESTRYMAP, however, provides estimates of linkage at loci between the genotyped markers and thus a more precise estimate of the location of a susceptibility locus. The results of both methods were consistent with each other with regard to the location of peak lod scores within the genotyped markers.

After data cleaning, there were 520 cases and 287 controls eligible for inclusion in the data. Reasons for exclusion included poor genotype quality and duplicate samples (several men had apparently enrolled in >1 of 3 Howard University studies that provided blinded samples).

Results from both ADMIXMAP and ANCESTRYMAP confirm linkage to the region on chromosome 8p (Supporting Data, Figure 1) detected by Freedman et al.(1). Using ADMIXMAP, the peak lod score on chromosome 8 of 2.37 was identified at marker rs4367565. ANCESTRYMAP identified the peak lod score on chromosome 8 of 1.543 between rs12547950 and rs4367565. Results from both methods are consistent with findings of Freedman et al. (1) and several subsequent confirmatory studies (6-11).

Additionally, linkage to a new region on chromosome 5q35 (Supporting Data, Figure 2) was detected in our study population. Using ADMIXMAP, the peak lod score was 3.16 at marker rs7729084. Similarly, the peak lod score on chromosome 5 identified by ANCESTRYMAP was 2.07 at this same marker. We are in the process of identifying candidate genes in this region and also preparing to perform fine mapping in an independent sample of prostate cancer cases and controls.

We are currently running admixture mapping analyses stratifying by age at diagnosis and prostate cancer stage and grade for inclusion in our manuscript.

Task 3. Final Analyses and Report Writing, Months 20-24:

- a. A final report describing the mapping findings and any gene-environment interactions will be prepared (months 20-24).*

Preliminary findings were presented in an abstract (see Appendix) and poster at the 2007 IMPaCT meeting in Atlanta, GA. The genomewide results are summarized in Figure 3 (see Supporting Data). The final manuscript is currently being prepared for submission to a peer-reviewed journal.

KEY RESEARCH ACCOMPLISHMENTS:

- Genomewide genotype data was generated for xx prostate cancer cases and xx controls.
- Genomewide admixture mapping was performed on these samples using ANCESTRYMAP and ADMIXMAP programs.
- A prostate cancer susceptibility locus on chromosome 8q24 was confirmed.
- A new prostate cancer susceptibility locus on chromosome 5q35 was identified, with a maximum lod score of 3.16 detected at marker rs7729084.
- Preliminary results were presented at the 2007 IMPaCT meeting in abstract and poster form.

REPORTABLE OUTCOMES:

A database of genotype information on 745 African American men has been established. Preliminary findings were presented in an abstract (see Appendix) and poster at the 2007 IMPaCT meeting in Atlanta, GA. The genomewide results are summarized in Figure 3 (see Supporting Data). The final manuscript is currently being prepared for submission to a peer-reviewed journal.

CONCLUSION:

The presence of a prostate cancer susceptibility locus on 8q24 was confirmed, with a peak lod score of 1.54 between markers rs12547950 and rs4367565 estimated using ANCESTRYMAP. A new prostate cancer susceptibility locus on chromosome 5q35 was identified, with a maximum lod score of 3.16 detected at marker rs7729084 using the ADMIXMAP statistical program. This new region is actively being explored via fine mapping and candidate gene approaches.

PERSONNEL:

Cathryn Bock
Wei Chen
Elisabeth Heath
Paul McKeigue (Consultant)
Benjamin Rybicki

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APPENDIX: IMPaCT abstract

PROSTATE CANCER GENE IDENTIFICATION BY ADMIXTURE MAPPING IN AFRICAN AMERICAN MEN

Cathryn H. Bock, Ann G. Schwartz, David Reich (Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, MA), Susan J. Land, Rick A. Kittles (University of Chicago, Chicago, IL), Benjamin A. Rybicki (Henry Ford Health System, Detroit, MI)

Evidence for a genetic component to prostate cancer risk is strong, however few genes have been identified, and most of the genetic risk remains undefined. To date, multiple traditional genome scans and linkage analyses have been performed, and several susceptibility loci and candidate genes have been identified. This research project used a novel approach to gene discovery, admixture mapping, to identify potential prostate cancer susceptibility genes in a sample of African American men from two case-control studies of prostate cancer. The admixture mapping method has greater power to detect genetic effects than traditional genome linkage scans. Recently, Freedman et al. published results from an admixture mapping study of prostate cancer in 1,597 African American men which detected a susceptibility region on chromosome 8q24 (*PNAS* 103(38) 14068-73). In the current study, samples from 520 cases and 287 controls have been genotyped for 1,536 ancestry informative markers from across the genome, using an updated version of the marker panel used by Freedman et al. Genotyping was performed using a custom array on the Illumina BeadStation platform using GoldenGate assay technology. At the time of abstract submission, all genotyping has been completed and data cleaning and analyses using ANCESTRYMAP and ADMIXMAP software programs are underway; preliminary results will be available by the end of April. Regions showing strong linkage using the admixture mapping approach will be followed up using fine mapping with a denser set of informative markers in the regions of interest and/or candidate gene studies. The goal of this project is to identify genomic prostate cancer susceptibility loci in a sample of African American men which can then lead to the identification of genes associated with increased prostate cancer risk; ultimately, this genetic information will be used to identify high risk individuals, and will improve screening techniques and prevention targeting efforts.

SUPPORTING DATA:

Figure 1: Admixture mapping results, chromosome 8

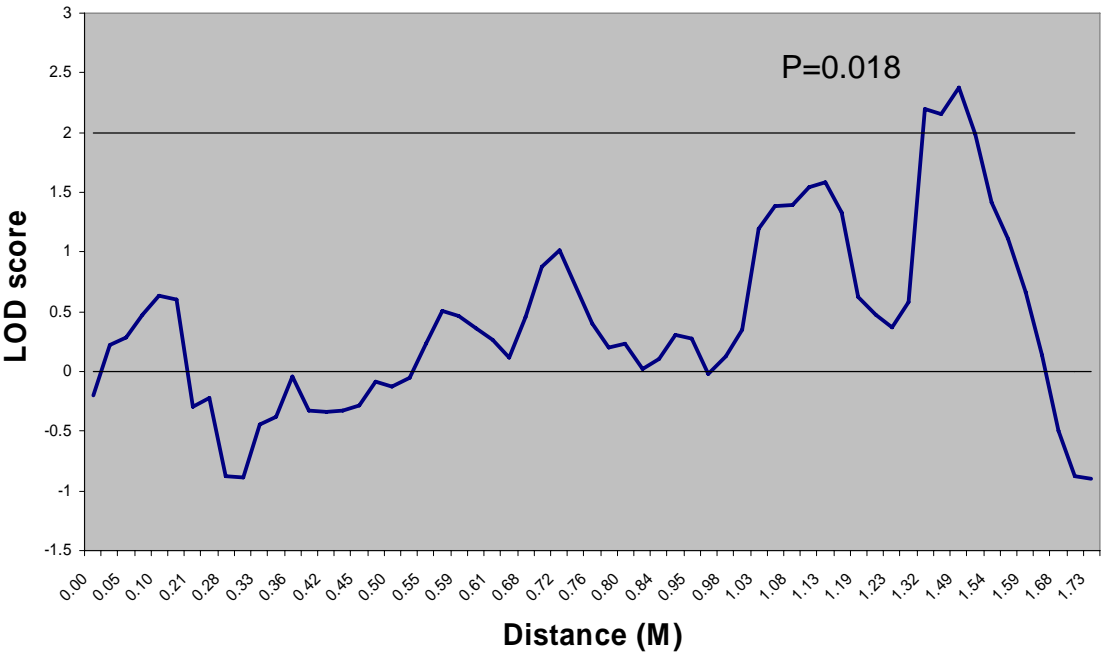


Figure 2:
Admixture mapping results, chromosome 5

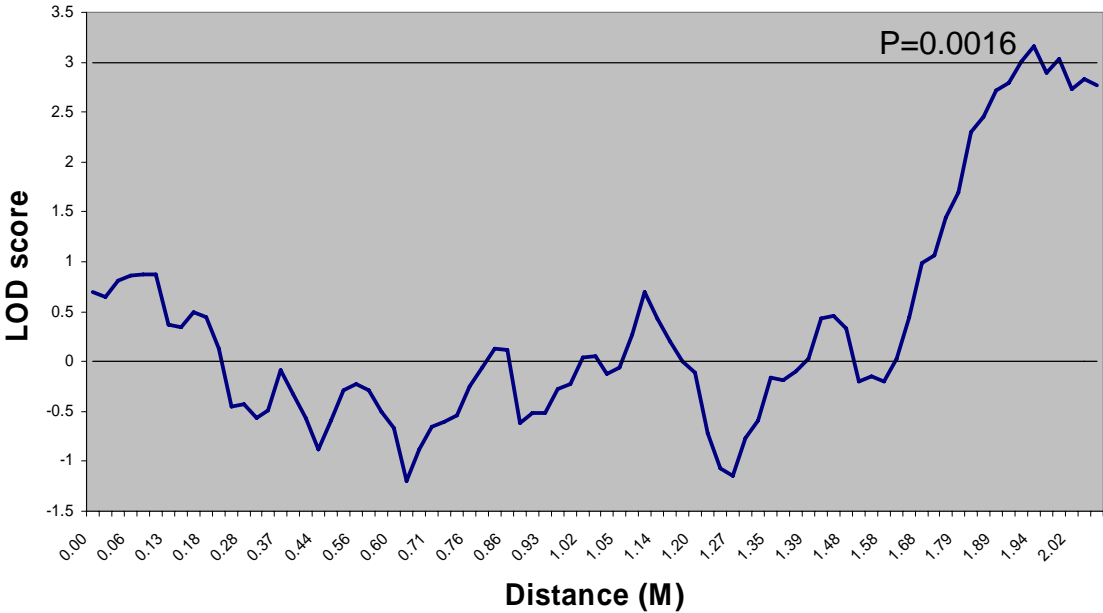


Figure 3: Summary of ANCESTRYMAP Results for a Whole Genome Admixture Scan

